Monomorphism in humans and sequence differences among higher primates for a sequence tagged site (STS) in homeo box cluster 2 as assayed by denaturing gradient electrophoresis

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The human homeo box cluster 2 (HOX2) contains genes coding for DNA binding proteins involved in developmental control and is highly conserved between mouse and man [1]. We have applied in concert the **Polymerase Chain Reaction (PCR)** and **Denaturing Gradient Electrophoresis (DGE)** to amplify defined primate HOX2 segments and to detect sequence differences among them. If priming sites are conserved, homologous PCR products can be amplified from primate genomes via **Cross-Species PCR (CS-PCR)** using human primers [2]. When subject to DGE, sequence differences in the most labile domain of CS-PCR products will change the gel position at which the molecules partially melt and stop migration [3].

We have sequenced a PstI fragment 4 kb upstream from HOX 2.2 and synthesized primers delimiting both halves of a 630 bp segment within it [4]. PCR on various unrelated humans and CS-PCR on chimpanzee, gorilla, orangutan and gibbon yielded products of the same length for each primer pair (Fig. 1a, c). The distal half, 'Pyg 3−2', is 300 bp long and the proximal, 'Pyg 1−4', is 320 bp. The 630 bp segment and flanking primers (Pyg 1, 2 below) define a **Sequence Tagged Site (STS)**[5] for the HOX2 region on human chromosome 17 (17q21-q22). Nested oligonucleotides (Pyg 3, 4 below) can serve as probes for confirming the STS.

All products focus on DGE at 2.5−3.1 M urea, 14−18% formamide (Fig. 1b, d). No polymorphism is detected in humans for either half. In Pyg 3−2, gorilla melts at 1% denaturant less than human; chimpanzee, at 5% less (Fig. 1b). Hence, with respect to human, chimpanzee difference(s) destabilize Pyg 3−2 more than gorilla difference(s). Orangutan and gibbon do not amplify, presumably due to mismatch at priming sites. For Pyg 1−4, gorilla and chimpanzee melt at about the same range as humans (Fig. 1d). Orangutan and gibbon are least stable: orangutan melts at 1% denaturant less and gibbon at 5% less than the other primates.

PCR/DGE analysis was carried out for a total of 26 unrelated humans from 17 ethnic groups. No sequence polymorphism was uncovered, rendering this locus monomorphic at the resolution afforded by DGE. However, a rare 100 bp insertion in Pyg 1−4 was observed in the Pygmy population (data not shown).

Detection of sequence differences among primates by CS-PCR/DGE can be useful for elucidating evolutionary relationships. Contrasting polymorphic frequencies among primates and humans for HOX2 may offer clues on functional genetic constraints and on evolutionary selection specific to each species.

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REFERENCES

1. Schughart *et al.* (1988) *Br. J. Cancer* **58 (Suppl IX)**, 9.
2. Ruano *et al.* (1989) *J. Cell Biochem.* **13E**, 303.
3. Lerman *et al.* (1984) *Ann. Rev. Biophys. Bioeng.* **13**, 399.
4. Ferguson-Smith (1989) PhD Thesis, Yale University.
5. Olson *et al.* (1989) *Science* **245**, 1434.
6. Saiki *et al.* (1988) *Science* **239**, 487.

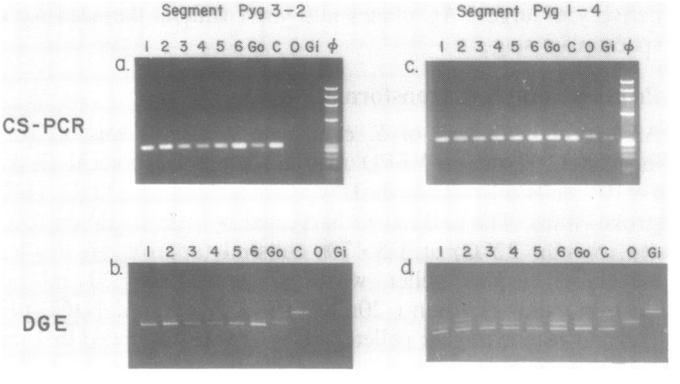

Figure 1. CS-PCR products from HOX2 segments for humans (1−6) and gorilla (Go), chimpanzee (C), orangutan (O) and gibbon (Gi). The survey of 26 humans included Caucasian (lane 1), Pygmy (2), San Bushman (3), Mayan (4), Cambodian (5), Thoti Indian (6) and others not shown: Druze, Ethiopian, Libyan, Bantu, Middle Eastern Arab, Chinese, Melanesian, Amerindian (Venezuelan and Brazilian), Japanese and Assamese. 15% of products was electrophoresed on 2.5% agarose (Panels a, c) and on a 30−70% denaturing gradient gel (Panels b, d) and photographed after EtBr staining. 3 melting domains are predicted for Pyg 1−4 by Lerman's Meltmap model [Ref. 3] (not shown) and probably account for minor bands in Panel d. 'ϕ' lanes contain 500 ng of ϕX174 DNA/HaeIII digest. PCR 30 cycles [1′ at 94°C; 1′ at 58°C (Pyg 3−2) or 55°C (Pyg 1−4); at 72°C]. **PCR** mixes as in Ref. 6 with 0.1 μM primers and 1μg genomic DNA. **Primers** (5′ to 3′) Pyg 1 AGTTCGGGAGTAAAATCTTG; Pyg 2 GCTCTATAGGAGGCCCTGAG; Pyg 3 GAGGCTGTTTAGATGA-GACA; Pyg 4 GGGAGTGATCACTCAGTACC [Ref. 4] **DGE** Linear gradient of urea(M)/formamide(%) denaturants ranged from 2.1 M/12% to 4.9 M/28% on 8% acrylamide, 1X TAE. Electrophoresis was at 80V for 15 hr, constant 60°C.